

1677-Pos Board B569**The Role of Notch Signaling in Mechanical-Tension Regulation of H2-Calponin Gene**

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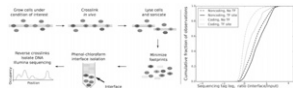
The essential role of mechanical signaling in regulating the cellular function of living organisms has been widely recognized. However, how mechanical signals are transduced in cells to regulate gene expression and other biochemical activities is not well understood. Our previous studies have demonstrated that the gene encoding h2-calponin (Cnn2) is regulated by mechanical tension (Hossain et al., JBC 280:42442-53, 2005). The data indicated that cis-regulatory element(s) located between -1.6-kb and -1.4-kb upstream of the mouse Cnn2 gene is responsible for the mechanical tension-regulation. The present study quantitatively studied the regulation of h2-calponin gene by mechanical tension in the cytoskeleton focusing on this region. Potential transcriptional regulatory factor binding sites within the -1.6 to -1.4-kb region were explored using reporter gene constructs in cells cultured on high and low stiffness substrates or in comparison between floating and adherent cultures. The results indicated a role of HES1 downstream of the Notch signaling pathway. While floating cultures of C2C12 myoblasts showed a significant decrease in h2-calponin expression compared to the adherent cultures, treatments with a Notch pathway inhibitor DAPT were able to minimize this low cytoskeleton tension-dependent inhibition of Cnn2 expression in a dose dependent manner. The Notch signaling pathway has been implicated in the mechanical tension-regulation of cells and our results suggest that it plays a role in the regulation of Cnn2 gene. The current hypothesis is that the Notch pathway modulates h2-calponin gene expression via the HES1 site in the -1.6kb and -1.4-kb region of the Cnn2 promoter where it acts to inhibit expression when cells are experiencing decreased mechanical tension. Further studies are underway to further elucidate the mechanotransduction mechanism that regulates h2-calponin expression.

1678-Pos Board B570**Genome-Wide Measurement of Bacterial Transcriptional Regulatory States**

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The regulation of gene expression plays a pivotal role in all aspects of biology, from the manner in which bacteria respond to their environment to the differentiation of tissues in higher eukaryotes. In the era of genomics, proteomics, and metabolomics, however, biologists are still bereft of a generally applicable method for rapid determination of the regulatory logic underlying the pattern of gene expression in a bacterial cell under a given set of conditions. This logic arises in large part from the binding of transcription factors (TFs) which can either repress or activate expression of nearby genes. We have recently developed a method enabling the simultaneous measurement of the complete regulatory state of bacterial cells under physiological conditions, in the form of a map of protein binding occupancies throughout the genome. This is accomplished by physically separating protein-DNA complexes from bulk genomic DNA, sequencing the footprinted complexes, and then applying a statistical mechanics-based model to identify protein occupancies. The newly developed method is applicable to any bacterium without the need for prior knowledge of its regulatory network, and will allow the rapid dissection of bacterial responses to environmental stresses.

**1679-Pos Board B571****Cellular Volume is a Global Controller of mRNA Abundance**

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The biochemical view of cellular function requires that the concentrations of cellular constituents remain the same for cells to function properly. However, cells in a population can vary widely in both volume and the numbers of these constituent molecules, implying that densities must fluctuate widely from cell to cell, and it remains an open question to understand how cells can function in the presence of this molecular "noise". Thus, we measured mRNA density in single cells using RNA fluorescence in situ hybridization (RNA-FISH). We find that many different species of mRNA in a given cell line exhibit constant density across a 4- to 6-fold volume range, each with their own characteristic density. We further found that quiescent and senescent cells also maintained the same characteristic density, despite changes in mean mRNA number. Thus, for many genes, RNA abundance is not random but precisely controlled to produce the appropriate amount of mRNA given the size of the cell, as though genes know how big the

cell is. There must therefore exist some density conservation mechanism. We hypothesize that mRNA density is maintained due to increased transcription in larger cells. Using RNA-FISH to visualize transcription at the single-gene level, we observe that overall transcriptional activity indeed scales with volume. We present a model explaining which transcriptional parameters govern density conservation despite transcription occurring in random bursts. Our findings suggest that global properties of RNA dynamics require a reassessment of our understanding of cellular heterogeneity and stochastic gene expression, and further suggest that evolution selects for particular genes to have certain transcriptional parameters in order to maintain their density. Our results suggest that density conservation is a natural consequence of the global feedback between cell volume and mRNA abundance independent of any specific mechanism.

1680-Pos Board B572**IFN-Independent Expression of RIG-I is a Determinant of Heterogeneous IFN-B Expression States in Antiviral Signaling**Sultan Doganay¹, Maurice Youzong Lee¹, Alina Baum²,Sun-Young Hwang³, Joo-Yeon Yoo³, Sua Myong¹, Adolfo Garcia Sastre⁴, Taekjip Ha¹.

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RIG-I plays a major role in antiviral innate immunity by detecting the cytoplasmic viral RNA and triggering the pathway that leads to transcriptional activation of type I interferon (IFN). Type I IFN induces a large set of genes called interferon stimulated genes (ISGs) which coordinate to antagonize viral evasion. We utilized single-molecule fluorescence in situ hybridization (smFISH) to study viral and antiviral gene expression in individual cells. We precisely quantified kinetics of mRNA expression of RIG-I, IFN- β and Sendai virus (SeV) L gene in clonal mammalian cells upon infection with SeV. Contrary to the expectation that RIG-I expression is only up-regulated through positive feedback involving Type I IFN, we found that RIG-I is directly induced by viral infection in the absence of IFN at the early stages of viral infection. Examination of expression kinetics of a subset of ISGs showed the differential regulation of ISGs in response viral infection. MDA5, LGP2, OasL and Viperin were directly activated by SeV infection before IFN- β . On the other hand, activation of some other ISGs was found to be dependent on IFN signaling, such as PKR, MxA, IRF7, TRIM25, and NLRX1. smFISH experiments revealed two distinct populations of cells which would otherwise be obscured in ensemble measurements: IFN- β expressing and IFN- β non-expressing cells. Simultaneous counting of IFN- β , RIG-I and SeV L gene mRNA and correlation analysis in individual cells revealed that transcriptional activation of IFN- β is dependent on the level of RIG-I mRNA in individual cells that is IFN-independently induced at the early stages of viral infection but is independent of the level of viral replication.

1681-Pos Board B573**Predicting Rates of Cell State Change due to Stochastic Fluctuations using a Data-Driven Landscape Model**

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We develop a potential landscape approach to quantitatively describe experimental data from a fibroblast cell line that exhibits a wide range of green fluorescent protein (GFP) expression levels under the control of the promoter for tenascin-C. Time lapse live cell microscopy provides data about short term fluctuations in promoter activity, and flow cytometry measurements provide data about the long term kinetics as isolated subpopulations of cells relax from a relatively narrow distribution of GFP expression back to the original broad distribution of responses. The landscape is obtained from the steady state distribution of GFP expression and is connected to a potential-like function using a stochastic differential equation description (Langevin/Fokker-Planck). The range of cell states is constrained by a "force" that is proportional to the gradient of the potential, and biochemical noise causes movement of cells within the landscape. Analyzing the mean square displacement of GFP intensity changes in live cells indicates that these fluctuations are described by a single diffusion constant in log GFP space. This allows application of the Kramers' model to calculate rates of switching between two attractor states, and enables an accurate simulation of the dynamics of relaxation back to the steady state with no adjustable parameters. With this approach, it is possible to use the steady state distribution of phenotypes and a quantitative description of the short term fluctuations in individual cells to accurately predict the rates at which different phenotypes will arise from an isolated subpopulation of cells.